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Article type : Letter to the Editor

Direct evidence for local IgE production in the human colonic mucosa

To the Editor,

Understanding the biology of IgE in humans has become a matter of interest that remains incompletely understood due to the rarity of peripheral IgE^+ cells. The increased incidence of allergic diseases and food-induced anaphylaxis overtime demands an urgent development of disease-modifying therapies that reverse the synthesis of IgE and the induction of IgE-mediated allergic reactions. Although some reports showed specific IgE in stools and IgE^+ cells in the gastrointestinal tract of allergic patients,[1,2] the microanatomical location of the class-switch recombination (CSR) to ε chain is largely unknown. This isotype is produced through CSR mechanism by activated IgM-producing B cells (direct switch) or following IgG^+ B memory cell-switch to ε chain (sequential switch) in a Th2 milieu with the induction of the cytidine deaminase (AID).[3] It has been demonstrated that this mechanism occurs prior to germinal center (GC) formation in secondary lymphoid tissues such as lymph nodes and tonsils,[4] but itis controversial whether the IgE isotype switching can occur in the human intestinal mucosa and which niches could be involved. Our study aimed to investigate the local IgE synthesis in the stroma of juvenile colonic polyps (JP) from patients with rectal bleeding and the relationship between IgE production and food sensitization, a risk factor for food allergy.

Pediatric patients with rectal bleeding (n=54) assisted at the Gastroenterology Unit of the Children´s Hospital of La Plata (Buenos Aires, Argentina) were selected for this study. The video colonoscopy revealed juvenile polyps (JP) and nodular lymphoid hyperplasia (NLH) (n=26), NLH (n=23), Crohn´s disease (n=2), familial adenomatous polyposis (n=2), or no abnormality (n=1). JPs

were removed along with a biopsy of the surrounding tissue (SCT), and patients were also studied at the Allergy Unit to assess their atopic condition and sensitization to food allergens (skin prick test-SPT, serum IgE tests and anamnesis). The Ethics Committee of the Children´s Hospital of La Plata approved the study, and all participants or their parents provided written informed consent.

Patient characteristics are described in Table 1. Thirteen out of the 26 patients had a personal or family history of allergy, 92.3% (n=24) had high levels of serum cow's milk-, soy- or peanut-specific IgE, a median value of total IgE of 356.5 IU/ml in serum (IQR= 101.2-564.9 IU/ml) and a median count of blood eosinophils of 3% (IQR= 2-5%). SPT was assessed in all patients, and we found one positive response to milk extract. Since this child had previously shown an allergic reaction after milk consumption, it was diagnosed with a milk allergy.

The histological analysis by H&E staining showed that the stroma of JP had infiltration of mononuclear cells, eosinophils, and mast cells. Cell agglomerations compatible with germinal center-like structures were observed in 92.3% of polyps. Furthermore, we confirmed the presence of GC by the detection of specific markers (n=10):CD20 (B cells), ki-67 (proliferating cells), and AID (CSR and hypermutation) (Figure 1A). SCT showed no cell infiltration and faint IgE staining, whereas IgE was positive in polyp tissue (PT). Conversely, IgA was positive in SCT, and faint staining was revealed within the JP. Additionally, the confocal microscopy revealed an augmented frequency of IgE^+ plasma cells in PT (40.5±6.71% vs. 2.05±0.50% IgE^+CD138^+ cells of total plasma cells in PT and SCT, respectively) (Figure 1B) and IgE^+CD138^- cells with morphology compatible with the bilobed-nucleus of eosinophils (Figure S1A).

Cytokines and soluble IgE were also quantified in tissue homogenates by ELISA. Our findings showed IL-4 and the IL-13/IFN-γ ratio higher in PT than in SCT (Figure 1C), and higher levels of total IgE in PT than in SCT. We found that total tissue IgE significantly correlated with serum total IgE level (Figure 1D), whereas a moderate negative correlation was observed with the frequency of JP´s eosinophils (Figure S1B), which may be due to the binding of soluble IgE to the high-affinity membrane receptor. Regarding tissue-specific IgE, we found higher levels of milk-specific IgE in PT than in SCT in 9 out of 26 patients. Soy- and peanut-specific IgE were not assessed in tissues because of limitations in the sample size.

Eight GC-like structures were isolated from PT by laser dissection microscopy (LDM) and RNA was purified. Three out of 8 GCs expressed AID mRNA (PL-24, 25 and 26) and were selected to study the intermediates of direct and sequential CSR by RT-PCR, according to Takhar *et al.* (Figure 1E).[5] SCT were also analyzed. Epsilon germline transcript (εGLT) was revealed in 1 out of 3 GCs analyzed, γ_1GLT and γ_3GLT transcripts were detected in 3 GCs and whole polyp tissues, whereas γ_4GLT was only detected in whole PT. The expression of the transient circle transcripts (CT)for a direct CSR from μ to ε chain (Iε-Cμ CT) was positive in2 GCs, while sequential CSR from γ to ε chains was only found in whole polyp tissues: Iε-Cγ1 CT in PL-25, Iε-Cγ3 CT in PL-24 and Iε-Cγ4 CT, in PL-26 (Figure 1E). No markers of CSR to IgE were found in SCT. Finally, the exon coding for mature ε was found in all GCs and PT, and IgE was also revealed in tissues of patients (Figure S1C). Epsilon exon and IgE were not detected in the SCT studied.

The literature shows that IgE synthesis studies were done in respiratory mucosa of patients with allergic rhinitis, and markers of direct and sequential CSR transcripts (Iε-Cμ CT and Iε-Cγ1/Iε-Cγ3/Iε-Cγ4 CT respectively)were detected in the mucosa or in allergen-stimulated explants.[5] Nevertheless, no studies have been performed in individual GCs. Although the retrospective study of Alexander *et al.* suggests a relationship between juvenile polyps and a personal or family history of allergy,[6] and the report by Roma-Giannikou*et al.*[7] described the presence of eosinophils in JP, there is no study showing an association between colorectal polyps, synthesis of IgE and food allergy or food sensitization. Recently, Boyd *et al.* described in peanut-allergic patients the existence of clonally related IgE^+ and non-IgE–expressing cells through DNA sequencing of the variable region of Ig heavy chain transcripts. Findings are indicative of local IgE CSR in the esophagus, stomach, and duodenum in which IgE and non-IgE cells of the same clones were found.[8] Interestingly, authors suggest that sequential CSR undergoes mainly from IgA1 to IgE, and IgG1 to IgE, as we report here and Curotto *et al*. described in mice.[9] Based on our findings, we propose that CSR to IgE can occur in the human colon without the implication of mesenteric lymph nodes, and as AID was expressed in GC, cells may also undergo somatic hypermutation. Although we could not evidence markers of CSR to IgE in all polyps, the significance of this study relies on the detection of intermediates of CSR to IgE in the human colon, which has not been reported in the literature. Since we found elevated levels of total and milk-specific IgE in polyps and serum, and there is a significant correlation between peripheral

and tissue total IgE, there could be an association between food sensitization and JP development.

In conclusion, our study showed direct evidence that the colorectal mucosa confined to colorectal polyps from patients sensitized to food allergens constitutes a tertiary lymphoid tissue containing active germinal centers with ongoing IgE synthesis through direct and sequential class-switch recombination mechanisms. Therefore, we suggest that there could be an association between rectal bleeding and colorectal polyps with allergen sensitization.

REFERENCES

1. Berin C, Wood S, Lopez-Exposito I, Sampson H, Nowak-Wegrzyn A. Detection Of Milk-specific IgE And IgA In Stool Samples From Children With Food Allergy. *J Allergy Clin Immunol*. 2011;127(2):AB181-AB181.

2. Hoh RA, Boyd SD. Gut Mucosal Antibody Responses and Implications for Food Allergy. *Front Immunol*. 2018;9:2221.

3. He J-S, Narayanan S, Subramaniam S, Ho WQ, Lafaille JJ, Curotto de Lafaille MA. Biology of IgE production: IgE cell differentiation and the memory of IgE responses. *Curr Top Microbiol Immunol*. 2015;388:1-19.

4. Roco JA, Mesin L, Binder SC, et al. Class-Switch Recombination Occurs Infrequently in Germinal Centers. *Immunity*. 2019;51(2):337-350.e7.

5. Takhar P, Smurthwaite L, Coker HA, et al. Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. *J Immunol*. 2005;174(8):5024-5032.

6. Alexander MRH, Beckwith JB, Morgan A, Bill AH. Juvenile polyps of the colon and their relationship to allergy. *Am J Surg*. 1970;120(2):222-225.

7. Papazoglou TA, Panayiotou JV, Vliet CPV, Kitsiou S. Colon Polyps in Childhood : Increased Mucosal Eosinophilia in Juvenile Polyps. 2008;21(4):229-232.

8. Hoh RA, Joshi SA, Lee J-Y, et al. Origins and clonal convergence of gastrointestinal IgE+ B cells in human peanut allergy. *Sci Immunol*. 2020;5(45).

9. Xiong H, Dolpady J, Wabl M, Curotto de Lafaille M a., Lafaille JJ. Sequential class switching is required for the generation of high affinity IgE antibodies. *J ExpMed*. 2012;209(2):353-364.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to the study:

-Conception and design of the study: GHD and CIM.

-Acquisition of data: KEC, MPM, MG, and EMA.

-Removal of the polyp: LG and VB.

-Analysis and/or interpretation of data: GHD, KEC, LG, VB, MG, and MEA.

-Drafting the manuscript: GHD.

-Revising the manuscript critically for relevant intellectual content: GHD, CIM, KEC, LG, and MG.

-Approval of the version of the manuscript to be published: GHD, CIM, KEC, MPM, LG, VB, MG, and MEA.

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KEC and MPM are fellows of CONICET; CIM and GHD are researchers of CONICET; LG and VB are gastroenterologists of the Children´s Hospital, MG is allergist of the Children´s Hospital, and EMA is pathologist of the Children´s Hospital.

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LEGEND TO FIGURE

FIGURE 1. Colorectal juvenile polyps show active germinal centers and local IgE synthesis. A, Histology by H&E staining(n=26) and immunohistochemistry (n=10) with anti-IgA, -IgE, -CD20, -Ki-67 or -AID antibody of polyp tissues (PT) and surrounding colonic tissues (SCT); germinal center-like structures were marked with a red dashed line. Representative images are depicted (patients PL-11 and PL-08 respectively). B, Protein concentration of IL-4 and IL-13/IFN-γ ratio in SCT and PT by ELISA. C, Confocal microscopy images (patient PL-10) showing CD138 (red), IgE (green) and propidium iodide (PI) for nucleus (blue); white arrows indicate $IgE^{+}CD138^{+}$ cells (n=10). D, Total and milk-specific IgE level by ELISA. Correlation between total IgE levels in PT and serum was calculated using Spearman's Rank-Order Correlation. E, Gel electrophoresis of RT-PCR-amplified fragments of intermediates of CSR from polyp-isolated germinal centers (GC) and its corresponding PT and SCT (representative image). L: 100-bp DNA ladder.

Graphics represent the mean and the SEM. Statistical differences were calculated using unpaired two-tailed *t*-tests. p-value and the number of samples analyzed are shown on the graphs.

TABLE1.Characteristics of pediatric patients.

Patient	Age (y)	Gender	Peripheral eosinophils (%)	Serum total IgE (IU/ml)	Serum cow's milk specific IgE [OD (Class)]	Serum soy specific IgE [OD (Class)]	Serum peanut specific IgE [OD (Class)]	SPT	Personal disease	Family clinical history
PL-01	6	M	5	9.3	0.197 (1)	0.351 (1)	0.537 (2)	(-)	Allergic rhinitis	Allergic rhinitis
PL-02	3	M	22	1007.0	0.309 (1)	0.234 (1)	0.109 (0)	(-)	No	No
PL-03	8	F	2	1193.0	0.279 (1)	0.307 (1)	0.356 (1)	(-)	Atopic dermatitis	No
PL-04	9	M	3	442.7	0.108 (0)	0.112 (0)	0.103 (0)	(-)	Allergic asthma	Allergic asthma
PL-05	4	M	3	549.0	0.287 (1)	0.581 (2)	2.468 (4)	(-)	No	No
PL-06	6	F	0	76.0	0.556 (3)	2.097 (4)	0.299 (1)	(-)	No	Allergic rhinitis
PL-07	6	M	6	0.0	0.097 (0)	0.107 (0)	0.074 (0)	(-)	No	No
PL-08	10	M	3	457.0	0.088 (0)	0.396 (1)	0.322 (1)	(-)	No	No
PL-09	10	F	3	1318.5	0.110 (0)	0.127 (1)	0.173 (1)	(-)	No	No
PL-10	0.5	M	1	59.3	0.368 (1)	0.105 (0)	0.391 (1)	(-)	Atopic dermatitis	No
PL-11	7	M	8	124.6	1.367 (4)	0.489 (2)	2.150 (4)	(-)	No	No
PL-12	5	M	2	547.5	0.099 (0)	0.212 (1)	0.386 (1)	(-)	Allergic asthma	Allergic asthma
PL-13	6	F	4	213.2	0.384 (1)	0.110 (0)	0.247 (1)	(-)	No	No
PL-14	3	M	9	525.2	0.354 (1)	0.094 (0)	0.111 (0)	(-)	No	Allergic asthma
PL-15	5	M	2	107.5	0.411 (2)	0.103 (0)	0.273 (1)	(-)	No	No
PL-16	6	F	2	238.7	0.399 (1)	0.113 (0)	0.636 (2)	(-)	No	No

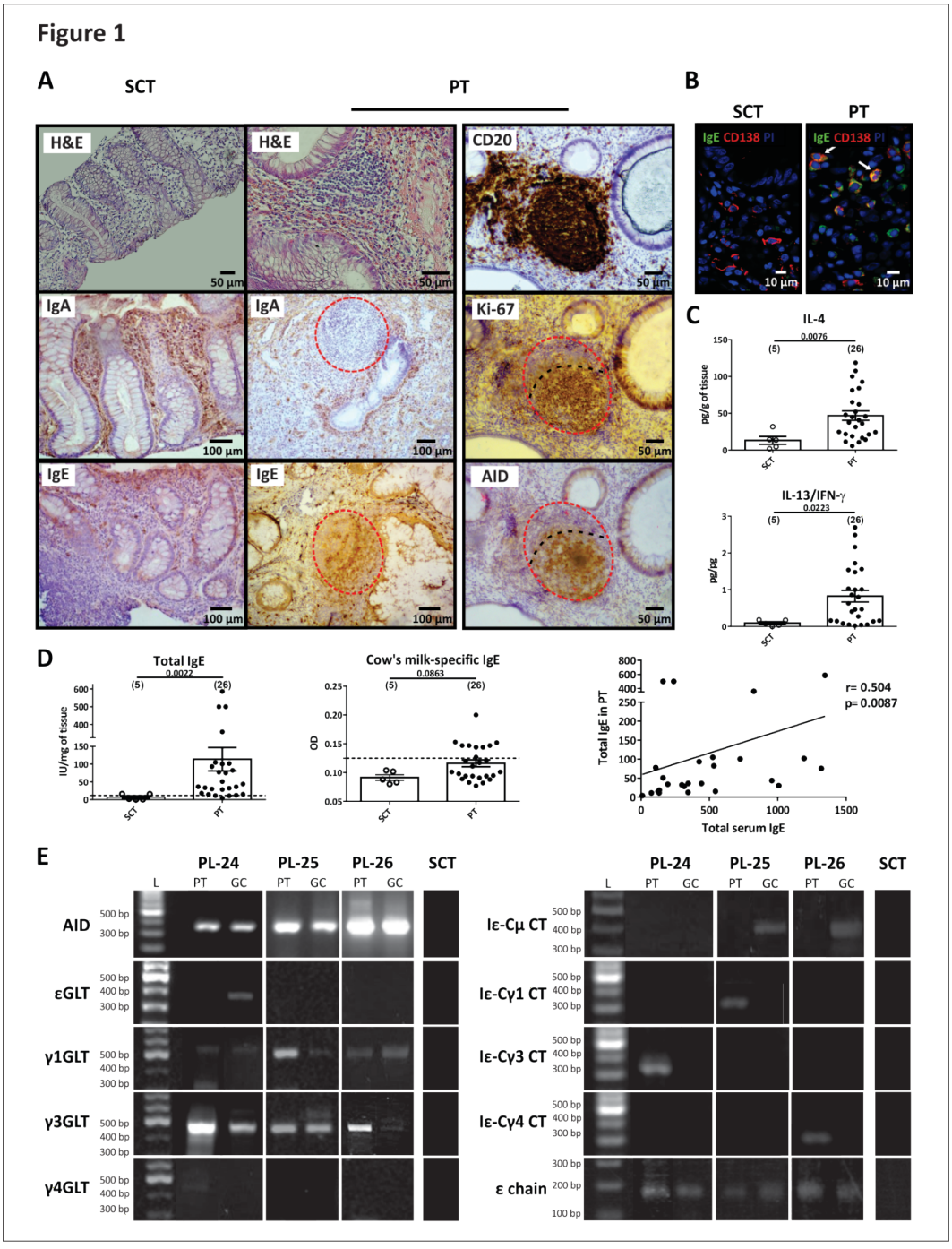
PL-17	4	F	5	628.6	0.274 (1)	0.531 (2)	0.709 (2)	(-)	No	Allergic rhinitis
PL-18	3	F	3	160.9	0.245 (1)	0.352 (1)	0.487 (2)	(+)(milk)	Cow's milk allergy	No
PL-19	3	F	2	75.2	0.201 (1)	0.467 (2)	0.459 (2)	(-)	No	No
PL-20	8	M	3	344.3	0.364 (1)	0.282 (1)	0.568 (2)	(-)	No	No
PL-21	9	F	2	132.2	0.227 (1)	0.112 (0)	0.102 (0)	(-)	No	No
PL-22	10	M	9	957.0	0.318 (1)	0.321 (1)	0.739 (3)	(-)	Allergic asthma	Allergic asthma
PL-23	5	M	2	544.3	0.473 (2)	0.421 (2)	0.804 (3)	(-)	Ant allergy	No
PL-24	3	M	3	716.2	0.427 (2)	1.526 (4)	1.143 (4)	(-)	No	Allergic rhinitis
PL-25	4	M	2	82.4	0.341 (1)	0.313 (1)	0.110 (0)	(-)	No	No
PL-26	4	M	2	368.7	1.268 (3)	2.272 (4)	2.963 (4)	(-)	Atopic dermatitis	Atopic dermatitis

IU/ml: International Units/ml.

OD: Optical density.

The cut-off OD for cow's milk, soy and peanut specific IgE is 0.113 and corresponds to0.35 kU_A/L.

Class: 0 corresponds to normal IgE level (below 0.35 kU_A/L) and class 1 to 4 correspond to IgE level ranging from 0.35 kU_A/L to 100 kU_A/L



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